

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER

P63132US0

US APPLICATION NO. (If known, see 37 CFR 1.5)

INTERNATIONAL APPLICATION NO.

PCT/EP97/02012

INTERNATIONAL FILING DATE

22 April 1997

PRIORITY DATE CLAIMED

22 April 1996

TITLE OF INVENTION

**BIOLOGICALLY ACTIVE PROTEIN (COLLAGEN FRAGMENT HF-COLL-18/514cf) FOR  
INHIBITING THE GROWTH OF TUMORS AND CAPILLARY PROLIFERATIONS**

09/171607

APPLICANT(S) FOR DO/EO/US

**Wolf-Georg FORSSMANN, Michael SCHRADER, Ludger STANDKER, Manfred RAIDA, Peter SCHULTZ-KNAPPE**

Applicant herein submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☒ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11. to 16. below concern other document(s) or information included:**

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.  
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

International Search Report — EPO

PCT/IB/304 Form

PCT/IB/308 Form

First Page of Publication

International Preliminary Examination Report in Translation — No Annexes

PCT/EP97/02012

P63132US0

17. ☒ The following fees are submitted:**Basic National Fee (37 CFR 1.492(a)(1)-(5)):**

Search Report has been prepared by the EPO or JPO ..... \$930.00

International preliminary examination fee paid to USPTO (37 CFR 1.482)  
..... \$720.00No international preliminary examination fee paid to USPTO (37 CFR 1.482)  
but international search fee paid to USPTO (37 CFR 1.445(a)(2)) ..... \$790.00Neither international preliminary examination fee (37 CFR 1.482) nor  
international search fee (37 CFR 1.445(a)(2)) paid to USPTO) ..... \$1,070.00International preliminary examination fee paid to USPTO (37 CFR 1.482)  
and all claims satisfied provisions of PCT Article 33(2)-(4) ..... \$98.00**ENTER APPROPRIATE BASIC FEE AMOUNT =**

CALCULATIONS

PTO USE ONLY

\$ 930.00

\$ 130.00

Surcharge of \$130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30  
months from the earliest claimed priority date (37 CFR 1.492(e)).

Claims	Number Filed	Number Extra	Rate
Total Claims	21 -20 =	-1-	X \$22.00
Independent Claims	1 -3 =	-0-	X \$82.00
Multiple dependent claim(s) (if applicable)			+ \$270.00

\$ 22.00

\$

\$

**TOTAL OF ABOVE CALCULATIONS =**

\$ 1082.00

Reduction by 1/2 for filing by small entity, if applicable.

Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).

\$

**SUBTOTAL =**

\$ 1082.00

Processing fee of \$130 for furnishing the English translation later the ☐ 20 ☐ 30  
months from the earliest claimed priority date (37 CFR 1.492(f)).**TOTAL NATIONAL FEE =**

\$ 1082.00

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be  
accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00

\$

**TOTAL FEES ENCLOSED =**

\$ 1082.00

Amount to be  
refunded:

charged:

a. ☒ A check in the amount of \$ 1082.00 to cover the above fees is enclosed.b. ☐ Please charge my Deposit Account No. 06-1358 in the amount of \$ --- to cover the above fees.  
A duplicate copy of this sheet is enclosed.c. ☒ The Commissioner is hereby authorized to charge my account any additional fees set forth in  
\$1.492 during the pendency of this application, or credit any overpayment to Deposit Account No.  
06-1358. A duplicate copy of this sheet is enclosed.

SEND ALL CORRESPONDENCE TO:  
**Jacobson, Price, Holman & Stern, PLLC**  
 400 7th Street, N.W.  
 Suite 600  
 Washington, DC 20004  
 202-638-6666

By William E. Player  
 William E. Player  
 Reg. No. 31,409

09/171607

800 Rec'd PCT/PTO 22 OCT 1998

PATENT

ATTY. DOCKET NO.: 10496/P63132USO

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:

FORSSMANN, et al.

Serial No.: National Stage of PCT/EP 97/02102 (22 April 1997)

Filed: Herewith

For: BIOLOGICALLY ACTIVE PROTEIN (COLLAGEN FRAGMENT HF-COLL-18/514cf)  
FOR INHIBITING THE GROWTH OF TUMORS AND CAPILLARY  
PROLIFERATIONS

**PRELIMINARY AMENDMENT**

Assistant Commissioner  
of Patents  
Washington, D.C. 20231

Sir:

Prior to calculating the filing fee, please amend the above-captioned application  
as follows.

**IN THE CLAIMS**

3. (Amended) A process for the preparation of the peptide according to  
claim 1 and/or its pharmacologically active fragments [according to claim 2],  
characterized in that it is prepared through prokaryotic or eukaryotic expression.

4. (Amended) A process for the preparation of the peptide according to claim 1 and/or its pharmacologically active fragments [according to claim 2], characterized in that it is isolated from human blood using chromatographic methods.

5. (Amended) A process for the preparation of the peptide or its derivatives according to claim 1 and/or its pharmacologically active fragments [according to claim 2], characterized in that said peptide or its derivatives or fragments are prepared from the amino acids contained in the stated sequence in protected form by common methods of solid-phase and liquid-phase synthesis, deprotected and purified by per se known chromatographical methods.

6. (Amended) Medicaments containing the peptide according to claim 1 or its pharmacologically active fragments [according to claim 2] as the active ingredient in addition to usual excipients and additives.

8. (Amended) Antibodies obtainable by immunizing animals with the peptide according to claim 1 and/or its pharmacologically active fragments [according to claim 2], and/or by using hybridoma technology.

10. A method for the treatment of patients in need of an inhibition of HF-COLL-18/514cf or its derivatives [or fragments] according to claim 1 [or 2] or its pharmacologically active fragments by the administration of therapeutic amounts of an antagonist/inhibitor of HF-COLL-18/514cf.

11. (Amended) Use of the medicaments according to [claims] claim 6 [or 7] for the treatment of diseases of the human organism, especially in connection with capillary proliferations.

12. (Amended) Use of the medicaments according to [claims] claim 6 [or 7] for the treatment of diseases of the human organism, especially carcinoses.

13. (Amended) Use of the medicaments according to [claims] claim 6 [or 7] for the treatment of diseases of the human organism, especially involving the cardiovascular and nervous systems.

14. (Amended) Use of the medicaments according to [claims] claim 6 [or 7] for the treatment of diseases of the human organism, especially involving the [intugement] integument and the sense organs, especially the eyes.

15. (Amended) Use of the peptide or its derivatives according to claim 1, [the] its pharmacologically active fragments [according to claim 2 or the antibody according to claim 8], or an antibody obtainable by immunizing an animal with said peptide and/or its pharmacologically active fragments and/or by using hybridoma technology for the preparation of a medicament for the treatment of disorders in inflammatory processes, disturbed inflammatory reactions, proliferation and maturation disorders of the blood-forming system.

16. (Amended) Use of the medicaments according to [claims] claim 6 [or 7 or the antibody according to claim 8] or an antibody obtainable by immunizing an animal with said peptide and/or its pharmacologically active fragments and/or by using hybridoma technology for the treatment of systemic diseases in an overproduction or deficiency of HF-COLL-18/514cf, especially when, e.g., antibodies have been formed against it in former applications, or the use of HF-COLL-18/514cf in substitution therapy.

17. (Amended) Use of the medicaments according to [claims] claim 6 [or 7] for the treatment of chronic diseases[, partially accompanied by the diseases

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mentioned in claims 11 to 16,] by using it in a suitable form for the treatment due to electrolytic activity in tumor and vascular diseases.

18. (Amended) Use of the medicaments according to [claims] claim 6 [or 7 or the antibody according to claim 8] or an antibody obtainable by immunizing an animal with said peptide and/or its pharmacologically active fragments and/or by using hybridoma technology for the treatment of acute diseases [as mentioned in claims 11 to 16] by using it in a suitable form for the treatment of these diseases in intensive care.

19. (Amended) Use of the medicaments according to [claims] claim 6 [or 7 of the antibody according to claim 8] or an antibody obtainable by immunizing an animal with said peptide and/or its pharmacologically active fragments and/or by using hybridoma technology for the diagnosis of diseases[, especially those mentioned in any of claims 11 to 16,] by preparing specific antibodies against synthetic fragments or the whole peptide or its derivatives and fragments and measuring the blood concentration of HF-COLL-18/514cf by immunoassays.

Serial No.: PCT/EP 97/02102 (22 April 1997)  
Atty. Docket No.: 10496/P63132US0

20. (Amended) A diagnostic agent containing the peptide according to claim 1, its pharmacologically active fragments [according to claim 2 or antibodies according to claim 8], or an antibody obtainable by immunizing an animal with said peptide and/or its pharmacologically active fragments and/or by using hybridoma technology for test systems for checking the levels of this substance in tissues, plasma, urine and cerebrospinal liquor.

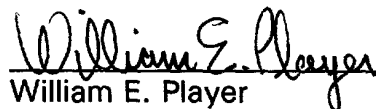
**R E M A R K S**

The present claims are 1-21.

By the instant Amendment the claims are rewritten to eliminate multiple dependencies and to more clearly define the instant invention.

Favorable action commensurate with the foregoing is requested.

Respectfully submitted,

  
William E. Player

Registration No. 31,409

JACOBSON, PRICE, HOLMAN & STERN, PLLC  
400 Seventh Street, N.W.  
Washington, D.C. 20004-2201  
Telephone: (202) 638-6666  
Atty. Docket No.: 10496/P63132US0  
Date: October 22, 1998

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Law Offices of  
**JACOBSON, PRICE, HOLMAN & STERN**  
PROFESSIONAL LIMITED LIABILITY COMPANY  
THE JENIFER BUILDING  
400 SEVENTH STREET, N.W.  
WASHINGTON, DC 20004

Attny's Docket No. 10496/P63132US

**SMALL ENTITY DECLARATION**  
[37 CFR 1.9(c-f)]

Each undersigned declares that:

(1) ☐ the application attached hereto.

(2) ☒ U.S. Application Serial No. 09/171,607, filed October 22, 1998

(3) ☐ U.S. Patent No. \_\_\_\_\_ Issued \_\_\_\_\_

is entitled to the benefits of "small entity" status for paying reduced fees under 35 USC 41(a) and (b) to the Patent and Trademark Office by virtue of the following:

(4) ☐ Each undersigned declares that he/she qualifies as an independent inventor, or would qualify had he/she made the as defined in 37 CFR 1.9(c).

(5) ☒ The undersigned declares that he/she is an official empowered to act on behalf of the concern identified below; that concern qualifies as a small business concern as defined in 37 CFR 1.9(d); that exclusive rights to the invention have been conveyed to and remain with the small business concern, or if the rights are not exclusive, that all other rights belong to small entities as defined in 37 CFR 1.9.

(6) ☐ The undersigned declares that he/she is an official empowered to act on behalf of the organization identified below, that organization qualifies as a nonprofit organization as defined in

(a) ☐ 37 CFR 1.9(e)(1)

(b) ☐ 37 CFR 1.9(e)(2)

(c) ☐ 37 CFR 1.9(e)(3)

(d) ☐ 37 CFR 1.9(e)(4) State law of \_\_\_\_\_ ;  
that exclusive rights to the invention have been conveyed to and remain with the organization, or if the rights are not exclusive, that all other rights belong to organizations as defined in 37 CFR 1.9.

(7) Each person, concern or organization to which I/we have assigned, granted, conveyed or licensed, or am under an under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

(a) ☐ no such person, concern or organization

(b) ☐ persons, concerns or organization listed below

[a separate declaration is required from each named person, concern or organization having rights to this invention averring to their status as "small entities."]

Full Name \_\_\_\_\_

Address \_\_\_\_\_

☐ Individual

☐ Small Business Concern

☐ Nonprofit Organization

I/we acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement of small entity prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I/we hereby declare all statements made herein of his/her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application, any patent issued thereon, or any patent to which this declaration is directed.

(8)

Typed Name of Inventor	Signature	Date
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

(9)

Name of Small Business Concern or Nonprofit Organization		
HaemoPep Pharma GmbH	By <u>[Signature]</u>	<u>Dec. 10, 1998</u>
Typed Name	Signature	Date
Klaus D. Döhler	_____	_____
Title of Signatory	_____	_____
Prof. Dr./General Manager	_____	_____

Law Offices of  
**JACOBSON, PRICE, HOLMAN & STERN**  
PROFESSIONAL LIMITED LIABILITY COMPANY  
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WASHINGTON, DC 20004

Attny's Docket No. 10496/P63132US

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(5) ☐ The undersigned declares that he/she is an official empowered to act on behalf of the concern identified below; that concern qualifies as a small business concern as defined in 37 CFR 1.9(d); that exclusive rights to the invention have been conveyed to and remain with the small business concern, or if the rights are not exclusive, that all other rights belong to small entities as defined in 37 CFR 1.9.

(6) ☐ The undersigned declares that he/she is an official empowered to act on behalf of the organization identified below; that organization qualifies as a nonprofit organization as defined in

(a) ☐ 37 CFR 1.9(e)(1)

(b) ☐ 37 CFR 1.9(e)(2)

(c) ☐ 37 CFR 1.9(e)(3)

(d) ☐ 37 CFR 1.9(e)(4) State law of \_\_\_\_\_ ;  
that exclusive rights to the invention have been conveyed to and remain with the organization, or if the rights are not exclusive, that all other rights belong to organizations as defined in 37 CFR 1.9.

(7) Each person, concern or organization to which I/we have assigned, granted, conveyed or licensed, or am under an under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

(a) ☐ no such person, concern or organization

(b) ☐ persons, concerns or organization listed below

[a separate declaration is required from each named person, concern or organization having rights to this invention averring to their status as "small entities."]

Full Name \_\_\_\_\_

Address \_\_\_\_\_

☐ Individual

☐ Small Business Concern

☐ Nonprofit Organization

I/we acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement of small entity prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I/we hereby declare all statements made herein of his/her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application, any patent issued thereon, or any patent to which this declaration is directed.

(8)

Typed Name of Inventor  
FORSSMANN, Wolf-Georg  
Typed Name of Inventor

Signature  
*W. G. Forssmann*  
Signature

Date  
*December 10, 1998*  
Date

Typed Name of Inventor

Signature

Date

Typed Name of Inventor

Signature

Date

(9)

Name of Small Business Concern or Nonprofit Organization

By  
Typed Name

Signature

Date

Title of Signatory

09/171607

300 Rec'd PCT/PTO 22 OCT 1998

SMB

A Biologically Active Protein - Collagen Fragment  
HF-COLL-18/514cf - for Inhibiting Tumor Growth  
and Capillary Proliferations

The present invention relates to a peptide (protein) which is capable of affecting the growth of cells. Collagen fragment HF-COLL-18/514cf and fragments and/or derivatives thereof as well as a medicament containing the natural and synthetic peptides can be employed for diagnostic or therapeutic purposes.

The invention relates to a process for obtaining a protein in a pure or partially purified form from human body fluids which protein is capable of affecting the growth of cells in an astonishing way, thereby inhibiting vascular and tumor growth. A similar substance has recently been detected in mice (O'Reilly et al., 1997, Cell, Vol. 88, page 277). The present substance, in contrast, is characterized in that it can be recovered, in particular, from hemofiltrate or hemodialyzate filtered from human blood. The substance has been designated HF-COLL-18/514cf and may be used for (1) analyzing diseases and (2) as a medicament.

The substance HF-COLL-18/514cf was first obtained from the hemofiltrate of patients suffering from renal diseases after ultrafiltration with a hemodialysis device, and characterized in terms of its molecular mass and the 60 N-terminal amino acids. For the preparation of HF-COLL-18/514cf, a patented process (Forssmann, 1988; Offenlegungsschrift DE 36 33 707 A1) has been sophisticated which had been invented for recovering proteins from hemofiltrate. Among the molecules obtained by this process having a molecular weight of below 20 kilodalton which are

filtered off in veno-venous or arterio-venous shunting, the fractions containing the HF-COLL-18/514cf can surprisingly be recognized by mass spectrometry. It has further been found in other specialized processes that this substance could astonishingly be purified until a homogeneous protein was finally identified and its structure elucidated. Surprisingly, this substance is the fragment of a protein which to date has only been known on the cDNA level (Oh et al., 1994, Genomics, Vol. 19, page 494). The value of this invention is characterized in that this substance can be purified from hemofiltrate, which had been considered worthless, to be used as an economically utilizable substance.

Thus, a compound has been isolated the structure of which had been unknown and the site of formation of which in the body is still unclear. The therapeutic and economic use has been tested, and HF-COLL-18/514cf has surprisingly been recognized as an important circulating peptide of human blood.

The substance mentioned, HF-COLL-18/514cf, can be obtained by chemical synthesis and by genetic engineering and may be used for numerous other purposes, inter alia, for analysis in human blood as a pathognomonic diagnostic feature of diseases of vascular growth, of tumor growth, and of metastases.

Thus, the present invention relates to a novel peptide, HF-COLL-18/514cf, its preparation, medicaments containing it as well as formulations containing it and its use for preparing them, as well as its natural and pharmacologically compatible derivatives, especially amidated, acetylated, phosphorylated and glycosylated HF-COLL-18/514cf derivatives and fragments of this peptide. An average molecular weight of 18494 u dalton could be determined by mass spectrometry.

The blood peptide HF-COLL-18/514cf has the following amino acid sequence:

Val-Ala-Leu-Asn-Ser-Pro-Leu-Ser-Gly-Gly-Met-Arg-Gly-Ile-Arg-Gly-  
Ala-Asp-Phe-Gln-Cys-Phe-Gln-Gln-Ala-Arg-Ala-Val-Gly-Leu-Ala-Gly-  
Thr-Phe-Arg-Ala-Phe-Leu-Ser-Ser-Arg-Leu-Gln-Asp-Leu-Tyr-Ser-Ile-  
Val-Arg-Arg-Ala-Asp-Arg-Ala-Ala-Val-Pro-Ile-Val-Asn-Leu-Lys-Asp-  
Glu-Leu-Leu-Phe-Pro-Ser-Trp-Glu-Ala-Leu-Phe-Ser-Gly-Ser-Glu-Gly-  
Pro-Leu-Lys-Pro-Gly-Ala-Arg-Ile-Phe-Ser-Phe-Asp-Gly-Lys-Asp-Val-  
Leu-Arg-His-Pro-Thr-Trp-Pro-Gln-Lys-Ser-Val-Trp-His-Gly-Ser-Asp-  
Pro-Asn-Gly-Arg-Arg-Leu-Thr-Glu-Ser-Tyr-Cys-Glu-Thr-Trp-Arg-Thr-  
Glu-Ala-Pro-Ser-Ala-Thr-Gly-Gln-Ala-Ser-Ser-Leu-Leu-Gly-Gly-Arg-  
Leu-Leu-Gly-Gln-Ser-Ala-Ala-Ser-Cys-His-His-Ala-Tyr-Ile-Val-Leu-  
Cys-Ile-Glu-Asn-Ser-Phe-Met-Thr-Ala-Ser

The peptide HF-COLL-18/514cf provided by the present invention is now a readily available drug with the biological and therapeutic activity of a natural analogue of the substance occurring in blood.

The present invention provides a production process for said HF-COLL-18/514cf as well as the use of HF-COLL-18/514cf as a medicament for various therapeutic and diagnostic indications. HF-COLL-18/514cf may be used as a high-purity material, or in a partially purified mixture of peptides if this is sufficient for the particular use.

The peptide according to the invention, its derivatives and fragments can be prepared by various processes, e.g., through prokaryotic or eukaryotic expression and optionally chromatographic purification. It can further be isolated from human blood, e.g., by per se known chromatographic methods. Finally, HF-COLL-18/514cf or its derivatives or fragments can be prepared from the amino acids contained in the stated sequence in protected form by common methods of solid-phase and liquid-phase synthesis. After deprotecting, it can be purified by common chromatographical methods.

The medicinal formulation according to the invention contains HF-COLL-18/514cf or a physiologically compatible salt of HF-COLL-18/514cf. The form and composition of the medicament which contains the HF-COLL-18/514cf depends on the route of administration. Human HF-COLL-18/514cf can be administered parenterally, intranasally, orally, intravenously, intramuscularly, intracutaneously, intrathecally, locally-topically or transpulmonarily. Preferably, HF-COLL-18/514cf is manufactured into an injection preparation, either as a solution or as a lyophilizate to be dissolved immediately prior to use. The medicinal formulation may additionally contain additives which are required by the filling technique, contribute to solubility, stability or sterility of the medicament, or increase the efficiency of intake into the body. It is particularly advantageous to use the lyophilized form taken up with mannite in sterile ampoules to be dissolved in physiological saline and/or infusions for repeated individual injection and/or permanent infusion in amounts of 30  $\mu$ g to 30 mg of pure HF-COLL-18/514cf per unit dose.

The daily dose of HF-COLL-18/514cf to be administered depends on the indication and on the particular derivatives used. With i.v./i.m. injection, it is in the range of from 100 to 1200 units ( $\mu$ g)/day, and with daily subcutaneous injection, it preferably ranges from 300 to 2400 units ( $\mu$ g)/day.

The peptide HF-COLL-18/514cf according to the invention is characterized in that it is particularly suitable for long-term therapy of tumor diseases or other diseases which are characterized by uncontrolled vascular growth, and that it does not trigger an immune response in permanent treatment. The preparation according to the invention is particularly suitable for a combination therapy involving chemotherapy and radiotherapy, or subsequent to chemotherapy or radiotherapy in cancer.

The preparation according to the invention can further be employed as an agent for therapy and diagnosis in vascular diseases of the supporting and connective tissue, the respiratory tract,

the cardiovascular system and the urogenital system, the nervous system and the eyes since it can be used for the preparation of human-compatible antibodies which are suitable for detecting and affecting changes of vascular growth in these organs.

Such antibodies are basically obtainable by immunizing animals with the peptide according to the invention and/or its fragments, or by using hybridoma technology.

The present invention also relates to a process for the treatment of patients in need of HF-COLL-18/514cf or its derivatives or fragments by the administration of therapeutic amounts of HF-COLL-18/514cf. Patients suffering from overproduction of HF-COLL-18/514cf or its derivatives or fragments require the administration of therapeutic amounts of an antagonist/inhibitor of HF-COLL-18/514cf.

The medicament according to the invention is suitable for the treatment of diseases of the human organism, especially in connection with capillary proliferations, carcinoses, diseases involving the cardiovascular and nervous systems, diseases involving the intugement and the sense organs, especially the eyes.

According to the invention, there is claimed the use of the peptide or its derivatives, the fragments or the antibody according to the invention for the preparation of a medicament for the treatment of disorders in inflammatory processes, disturbed inflammatory reactions, proliferation and maturation disorders of the blood-forming system, of systemic diseases in an overproduction or deficiency of HF-COLL-18/514cf, especially when, e.g., antibodies have been formed against it in former applications, or the use of HF-COLL-18/514cf in substitution therapy, chronic diseases, partially accompanied by the diseases mentioned by using it in a suitable form for the treatment due to electrolytic activity in tumor and vascular diseases.

The medicament according to the invention is suitable for the treatment of acute diseases of the kinds mentioned above by using it in a suitable form for the treatment of these diseases in intensive care.

A further use of the peptide according to the invention, its fragments or the antibody according to the invention is for the diagnosis of diseases by preparing specific antibodies against synthetic fragments or the whole peptide or its derivatives and fragments and, e.g., measuring the blood concentration of HF-COLL-18/514cf by immunoassays.

Thus, a diagnostic agent containing the peptide according to the invention, its fragments or antibodies according to the invention for test systems for checking the levels of this substance in tissues, plasma, urine and cerebrospinal liquor is also a subject matter of the invention. The diagnostic agent according to the invention is particularly suitable as a marker for certain carcinoses and for functional disorders of blood vessels, bone marrow, lymph organs, the gastro-intestinal tract, the immune system and for inflammatory and neoplastic processes.

The invention will be further explained by means of the following Examples.

Example 1: Isolation and Characterization of Circulating HF-COLL-18/514cf from Human Hemofiltrate

As the starting material, there was used hemofiltrate which is obtained in large amounts in the treatment of renal insufficiency patients and contains all plasma components up to a molecular size of about 20,000 dalton.

I. Recovery of the raw peptide material

The hemofiltrate was obtained using a Sartorius hemofiltration plant and cellulose triacetate filters with an exclusion size of



20,000 dalton (SM 40042, Sartorius, Göttingen, Germany). The filtrate was derived from renal insufficiency patients which were in a stable metabolic condition from long-term hemofiltration, and protected from proteolytic degradation immediately after recovery by acidification and cooling at 4°C. In four extractions with a cation exchange column (TSK SP 650(M), Merck, Darmstadt, Germany), 2860 l of hemofiltrate was processed. 93% of the pooled extracts were successively eluted from the above-mentioned column material by different buffers having different pH values. The raw fractions were subsequently subjected to freeze-drying.

## II. Preparative RP chromatography

500 mg out of 2200 mg of the last raw fraction was roughly separated by hydrophobicity by means of preparative RP chromatography. Fractions were collected from a PrepPak Cartridge with dimensions of 47 x 300 mm supplied by Waters. Fraction 31 was used for further purification.

Device:	BioCad HPLC (Perseptive Biosystems, Freiburg, Germany)
Column:	Waters PrepPak Cartridge 47 x 300 mm
Material:	Vydac, 300 Å, 15 - 20 µm
Eluent A:	water with 10 mM HCl
Eluent B:	methanol with 10 mM HCl
Gradient:	0 - 50% eluent B 28.57 min 50 - 95% eluent B 61.43 min 95% eluent B 5.71 min
Flow rate:	35 ml/min
Fractions:	50 ml or 1.43 min
Detection:	230 nm and 280 nm

## III. First analytical RP HPLC

Ultraviolet absorption during analytical RP chromatography of fraction 31 which had been obtained from the separation in figure 1. In a gradient on a Vydac column (10 x 250 mm, steel coat,

material: RP C18, 300 Å, 5 µm), a further separation could be achieved. The eluents were water with 0.1% by volume of trifluoroacetic acid, and acetonitrile with 0.1% by volume of trifluoroacetic acid.

Device: Kontron HPLC plant  
Column: Vydac, steel coat, 10 x 250 mm  
Material: Vydac RP-C18, 300 Å, 5 µm  
Eluent A: water with 0.1% by volume of trifluoroacetic acid  
Eluent B: acetonitrile with 0.1% by volume of trifluoroacetic acid  
Gradient: 0 - 60% eluent B 50 min  
60 - 80% eluent B 5 min  
80 - 0% eluent B 5 min  
Flow rate: 2 ml/min  
Fractions: 2 ml or 1 min  
Detection: 230 nm

#### IV. Detection of the molecular mass of HF-COLL-18/514cf by means of MALDI TOF mass spectrometry

With a MALDI mass spectrometer RBT II (Vestec/PerSeptive, Houston, Texas, USA), mass spectra of the purified native HF-COLL-18/514cf from the preparation in step III were measured using  $\alpha$ -cyano-4-hydroxycinnamic acid as the matrix. In fractions 45 and 46, peaks of the singly, doubly and triply protonated molecule could be seen with a molecular mass of about 18500 u. In addition, various minor components could be seen.

#### V. Second analytical RP-HPLC

In a final analytical RP chromatography of pooled fractions 45 and 46 which had been obtained from the separation in step III, highly purified HF-COLL-18/514cf could be isolated in fraction 25.

Device: Kontron HPLC (Kontron, Munich, Germany)  
Column: YMC, steel coat, 4.6 x 250 mm  
Material: YMC RP-C18, 300 Å, 5 µm  
Eluent A: water with 0.1% by volume of trifluoroacetic acid  
Eluent B: 80% acetonitrile, 20% water (v/v) with 0.1% by  
volume of trifluoroacetic acid  
Gradient: 0 - 30% eluent B 5 min  
30 - 80% eluent B 150 min  
80 - 100% eluent B 5 min  
100 eluent B 5 min  
Flow rate: 0.6 ml/min  
Fractions: manually collected  
Detection: 230 nm and 280 nm

#### VI. Determination of purity by capillary zone electrophoresis

5 µl of fraction 25 was directly used for measuring in capillary zone electrophoresis. The electropherogram shows only one peak and no other peaks from minor components. This result shows that high purity HF-COLL-18/514cf was present in the final stage of purification.

Device: P/ACE System 2000, Beckman Instruments GmbH,  
Munich, Germany  
Capillary: uncoated fused silica, 500 mm x 75 µm ID  
Buffer: 100 mM sodium phosphate, pH 2.5  
0.02% hydroxypropylmethylcellulose  
Temperature: 25°C  
Injection: 20 s, corresponding to 120 nl  
Run: 25 minutes  
Current: 80 µA, constant  
Detection: 200 nm

#### VII. Determination of the Molecular Mass of HF-COLL-18/514cf

Spectra could be obtained by means of MALDI TOF mass spectrometry on a Vestec BT II from the purified native HF-COLL-18/514cf from

fraction 25 in step V on two matrices ( $\alpha$ -cyano-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid). Peaks are found from the singly, doubly and triply protonated molecules. The molecular mass is determined to be  $18507 \text{ u} \pm 20 \text{ u}$ . Minor components cannot be seen.

For a more accurate determination of the molecular mass of the purified native HF-COLL-18/514cf, an additional mass spectrum was measured of fraction 25 of step V using an electrospray mass spectrometer (Sciex API III, Perkin Elmer, Langen, Germany). Peaks can be seen from the molecules with eight to eleven protonations. The average molecular mass of HF-COLL-18/514cf is determined to be  $18494 \text{ u} \pm 3 \text{ u}$ , the theoretical value being  $18496 \text{ u}$  (see VIII).

#### VIII. Determination of the aminoterminal amino acid sequence

By automated Edman sequencing with a Gas Phase Amino Acid Sequenator ABI 494 (Applied Biosystems, Perkin Elmer, Weiterstadt, Germany), the first 60 amino acids were determined. At the 21st position (Xxx), no amino acid was detected, as expected with cystein.

Val-Ala-Leu-Asn-Ser-Pro-Leu-Ser-Gly-Gly-Met-Arg-Gly-Ile-Arg-Gly-Ala-Asp-Phe-Gln-Xxx-Phe-Gln-Gln-Ala-Arg-Ala-Val-Gly-Leu-Ala-Gly-Thr-Phe-Arg-Ala-Phe-Leu-Ser-Ser-Arg-Leu-Gln-Asp-Leu-Tyr-Ser-Ile-Val-Arg-Arg-Ala-Asp-Arg-Ala-Ala-Val-Pro-Ile-Val

Thus, it has been established that the fragment is derived from collagen alpha 1 (XVIII), this protein having been known to date only on the cDNA level (Oh et al., 1994, Genomics, Vol. 19, page 494). The fragment starts at position 514 of the protein precursor, and the molecular mass shows that it ends at the last position but one of the precursor with the amino acid serine, i.e., is truncated by one lysine at the C terminus.

Example 2:        Study of the Biological Effectiveness of HF-COLL-  
18/514cf

By the method illustrated in Example 1, a larger amount of material of more than 0.1 mg of HF-COLL-18/514cf was isolated from human hemofiltrate. The highly pure HF-COLL-18/514cf was employed in endothelial cell proliferation assays for the determination of its biological function. For this assay, bovine capillary endothelial cells from the adrenal cortex of freshly slaughtered calves were cultured as described in the literature (Folkman et al., 1979, Proc. Natl. Acad. Sci. Vol. 76, page 5217).

The proliferation assay was performed as described in the literature (O'Reilly et al., 1997, Cell, Vol. 88, page 277). Thus, the bovine capillary endothelial cells were washed with PBS (phosphate buffered saline, pH 7.4) and suspended in 0.05% trypsin solution. A cell suspension with 25,000 cells per ml in DMEM medium containing 10% FCS (fetal calf serum) and 1% GPS (glutamine-penicillin-streptomycin) was incubated in gelatine-coated 24-well plates (0.5 ml per well) at 37°C and 10% CO<sub>2</sub>.

After 24 h, the medium was replaced by 0.5 ml of DMEM medium containing 5% FCS and 1% GPS and varying concentrations (from 0 to 1000 ng/ml final concentration) of the isolated high purity HF-COLL-18/514cf. After another 30 minutes of incubation, bFGF (basic fibroblast growth factor) was added to the mixtures to a final concentration of 1 ng/ml. After 72 h, the cell count in the mixtures was determined by crystal violet staining of the cells and measuring the absorption at 600 nm. The HF-COLL-18/514cf added to the bovine capillary endothelial cells inhibited the bFGF stimulated proliferation of those cells in a concentration-dependent way. Half-maximum inhibition of the proliferation in this assay was reached with a concentration of 200 ng/ml HF-COLL-18/514cf.

•

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Wolf-Georg Forssmann
- (B) STREET: Feodor-Lynen-Str. 31
- (C) CITY: Hannover
- (E) COUNTRY: Germany
- (F) POSTAL CODE: D-30625

(ii) TITLE OF INVENTION: A Biologically Active Protein -  
Collagen Fragment HF-COLL-18/514cf - for Inhibiting  
Tumor Growth and Capillary Proliferations

(iii) NUMBER OF SEQUENCES: 1

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30  
(EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 170 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single-stranded
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Val	Ala	Leu	Asn	Ser	Pro	Leu	Ser	Gly	Gly	Met	Arg	Gly	Ile	Arg	Gly
1				5					10					15	
Ala	Asp	Phe	Gln	Cys	Phe	Gln	Gln	Ala	Arg	Ala	Val	Gly	Leu	Ala	Gly
			20					25					30		
Thr	Phe	Arg	Ala	Phe	Leu	Ser	Ser	Arg	Leu	Gln	Asp	Leu	Tyr	Ser	Ile
		35					40					45			
Val	Arg	Arg	Ala	Asp	Arg	Ala	Ala	Val	Pro	Ile	Val	Asn	Leu	Lys	Asp
	50					55					60				
Glu	Leu	Leu	Phe	Pro	Ser	Trp	Glu	Ala	Leu	Phe	Ser	Gly	Ser	Glu	Gly
65					70					75				80	
Pro	Leu	Lys	Pro	Gly	Ala	Arg	Ile	Phe	Ser	Phe	Asp	Gly	Lys	Asp	Val
				85					90					95	
Leu	Arg	His	Pro	Thr	Trp	Pro	Gln	Lys	Ser	Val	Trp	His	Gly	Ser	Asp
			100					105					110		
Pro	Asn	Gly	Arg	Arg	Leu	Thr	Glu	Ser	Tyr	Cys	Glu	Thr	Trp	Arg	Thr
		115					120					125			
Glu	Ala	Pro	Ser	Ala	Thr	Gly	Gln	Ala	Ser	Ser	Leu	Leu	Gly	Gly	Arg
	130					135					140				
Leu	Leu	Gly	Gln	Ser	Ala	Ala	Ser	Cys	His	His	Ala	Tyr	Ile	Val	Leu
145					150					155					160
Cys	Ile	Glu	Asn	Ser	Phe	Met	Thr	Ala	Ser						
				165					170						



C L A I M S :

1. A peptide having the following amino acid sequence:

Val-Ala-Leu-Asn-Ser-Pro-Leu-Ser-Gly-Gly-Met-Arg-Gly-Ile-Arg-  
Gly-Ala-Asp-Phe-Gln-Cys-Phe-Gln-Gln-Ala-Arg-Ala-Val-Gly-Leu-  
Ala-Gly-Thr-Phe-Arg-Ala-Phe-Leu-Ser-Ser-Arg-Leu-Gln-Asp-Leu-  
Tyr-Ser-Ile-Val-Arg-Arg-Ala-Asp-Arg-Ala-Ala-Val-Pro-Ile-Val-  
Asn-Leu-Lys-Asp-Glu-Leu-Leu-Phe-Pro-Ser-Trp-Glu-Ala-Leu-Phe-  
Ser-Gly-Ser-Glu-Gly-Pro-Leu-Lys-Pro-Gly-Ala-Arg-Ile-Phe-Ser-  
Phe-Asp-Gly-Lys-Asp-Val-Leu-Arg-His-Pro-Thr-Trp-Pro-Gln-Lys-  
Ser-Val-Trp-His-Gly-Ser-Asp-Pro-Asn-Gly-Arg-Arg-Leu-Thr-Glu-  
Ser-Tyr-Cys-Glu-Thr-Trp-Arg-Thr-Glu-Ala-Pro-Ser-Ala-Thr-Gly-  
Gln-Ala-Ser-Ser-Leu-Leu-Gly-Gly-Arg-Leu-Leu-Gly-Gln-Ser-Ala-  
Ala-Ser-Cys-His-His-Ala-Tyr-Ile-Val-Leu-Cys-Ile-Glu-Asn-Ser-  
Phe-Met-Thr-Ala-Ser (HF-COLL-18/514cf)

and its natural and pharmacologically compatible derivatives, especially amidated, acetylated, phosphorylated and glycosylated derivatives.

2. Fragments of the peptide according to claim 1 which are pharmacologically active.
3. A process for the preparation of the peptide according to claim 1 and/or its fragments according to claim 2, characterized in that it is prepared through prokaryotic or eukaryotic expression.
4. A process for the preparation of the peptide according to claim 1 and/or its fragments according to claim 2, characterized in that it is isolated from human blood using chromatographic methods.

5. A process for the preparation of the peptide or its derivatives according to claim 1 and its fragments according to claim 2, characterized in that said peptide or its derivatives or fragments are prepared from the amino acids contained in the stated sequence in protected form by common methods of solid-phase and liquid-phase synthesis, deprotected and purified by per se known chromatographical methods.
6. Medicaments containing the peptide according to claim 1 or its fragments according to claim 2 as the active ingredient in addition to usual excipients and additives.
7. Medicaments according to claim 6 for oral, parenteral, intravenous, intramuscular, intracutaneous, intrathecal, intranasal and local-topical application as well as in the form of an aerosol for transpulmonary application.
8. Antibodies obtainable by immunizing animals with the peptide according to claim 1 and/or fragments according to claim 2, and/or by using hybridoma technology.
9. A method for the treatment of patients in need of HF-COLL-18/514cf or its derivatives or fragments according to claim 1 by the administration of therapeutic amounts of HF-COLL-18/514cf.
10. A method for the treatment of patients in need of an inhibition of HF-COLL-18/514cf or its derivatives or fragments according to claim 1 or 2 by the administration of therapeutic amounts of an antagonist/inhibitor of HF-COLL-18/514cf.
11. Use of the medicaments according to claims 6 or 7 for the treatment of diseases of the human organism, especially in connection with capillary proliferations.

12. Use of the medicaments according to claims 6 or 7 for the treatment of diseases of the human organism, especially carcinoses.
13. Use of the medicaments according to claims 6 or 7 for the treatment of diseases of the human organism, especially involving the cardiovascular and nervous systems.
14. Use of the medicaments according to claims 6 or 7 for the treatment of diseases of the human organism, especially involving the intugement and the sense organs, especially the eyes.
15. Use of the peptide or its derivatives according to claim 1, the fragments according to claim 2 or the antibody according to claim 8 for the preparation of a medicament for the treatment of disorders in inflammatory processes, disturbed inflammatory reactions, proliferation and maturation disorders of the blood-forming system.
16. Use of the medicaments according to claims 6 or 7 or the antibody according to claim 8 for the treatment of systemic diseases in an overproduction or deficiency of HF-COLL-18/514cf, especially when, e.g., antibodies have been formed against it in former applications, or the use of HF-COLL-18/514cf in substitution therapy.
17. Use of the medicaments according to claims 6 or 7 for the treatment of chronic diseases, partially accompanied by the diseases mentioned in claims 11 to 16, by using it in a suitable form for the treatment due to electrolytic activity in tumor and vascular diseases.
18. Use of the medicaments according to claims 6 or 7 or the antibody according to claim 8 for the treatment of acute diseases as mentioned in claims 11 to 16 by using it in a

suitable form for the treatment of these diseases in intensive care.

19. Use of the medicaments according to claims 6 or 7 or the antibody according to claim 8 for the diagnosis of diseases, especially those mentioned in any of claims 11 to 16, by preparing specific antibodies against synthetic fragments or the whole peptide or its derivatives and fragments and measuring the blood concentration of HF-COLL-18/514cf by immunoassays.
20. A diagnostic agent containing the peptide according to claim 1, fragments according to claim 2 or antibodies according to claim 8 for test systems for checking the levels of this substance in tissues, plasma, urine and cerebrospinal liquor.
21. The diagnostic agent according to claim 20 as a marker for certain carcinoses and for functional disorders of blood vessels, bone marrow, lymph organs, the gastro-intestinal tract, the immune system and for inflammatory and neoplastic processes.

A b s t r a c t

The invention relates to a peptide obtained from human blood, HF-COLL-18/514cf, the structure of which has been elucidated for the purpose of diagnostic, medicinal and industrial use as a medicament. The isolation of this novel peptide HF-COLL-18/514cf proves the existence of HF-COLL-18/514cf. The molecular shape of HF-COLL-18/514cf has been detected by mass spectrometry and amino-terminal sequencing; HF-COLL-18/514cf is a natural peptide which should be used for the treatment of numerous diseases associated with cell growth disorders, especially of endothelial cells and vessels, and in cancer, for example. Further, HF-COLL-18/514cf can be used in a pure form or as a natural raw extract for industrial purposes in the investigation of new cellular functions.

# DECLARATION AND POWER OF ATTORNEY U.S.A.

FOR ATTORNEYS' USE ONLY

ATTORNEYS' DOCKET NO.

ALL PATENTS, INCLUDING DESIGN

FOR APPLICATION BASED ON PCT, PARIS CONVENTION;

NON PRIORITY; OR PROVISIONAL APPLICATIONS

As a below named inventor, I declare that my residence, post office address and citizenship are stated below next to my name, the information given herein is true, that I believe that I am the original, first and sole inventor (if only one name is listed at 201-203, or on additional sheets attached hereto) of the subject matter which is claimed and for which patent is sought on the invention entitled:

**A Biologically Active Protein - Collagen Fragment HF-COLL-18/514f - for Inhibiting Tumor Growth and Capillary Proliferations**

which is described and claimed in: ☒ PCT International Application No. PCT/EP 97/02012 filed April 22, 1997

☐ the attached specification ☐ the specification in application Serial No. \_\_\_\_\_ filed \_\_\_\_\_  
(if applicable) and amended on \_\_\_\_\_

I hereby state that I have reviewed and understand the contents of the above-identified specifications, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed

Prior Foreign Application(s)

196 15 710.2  
(Number)

Germany DEX  
(Country)

22/04/1996  
(Day/Month/Year Filed)

Priority Claimed

☒ Yes ☐ No

(Number)

(Country)

(Day/Month/Year Filed)

☐ Yes ☐ No

(Number)

(Country)

(Day/Month/Year Filed)

☐ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Application No \_\_\_\_\_ Filing Date \_\_\_\_\_ Application No \_\_\_\_\_ Filing Date \_\_\_\_\_

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application

(Application Serial No)

(Filing Date)

(Status: patented, pending, abandoned)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys (Registration No.) to prosecute this application, receive and act on instructions from my agent, and transact all business in the Patent and Trademark Office connected therewith. HARVEY B. JACOBSON, JR. (20,851); D. DOUGLAS PRICE (24,514); JOHN CLARKE HOLMAN (22,769); MARVIN R. STERN (20,640); MICHAEL R. SLOBASKY (26,421); JONATHAN SCHERER (29, 851); STANFORD W. BERMAN (17,909); IRWIN M. AISENBERG (19,007); WILLIAM E. PLAYER (31,409)

SEND CORRESPONDENCE TO

**JACOBSON, PRICE, HOLMAN & STERN**  
PROFESSIONAL LIMITED LIABILITY COMPANY  
400 Seventh Street, N.W.  
Washington, D.C. 20004

DIRECT TELEPHONE CALLS TO:

(please use Attorney's Docket No.) (202) 638-6666

**JACOBSON, PRICE, HOLMAN & STERN**  
PROFESSIONAL LIMITED LIABILITY COMPANY

Inventor(s) name must include at least one unabbreviated first or middle name.

201	FULL NAME* OF INVENTOR	FAMILY NAME <u>FORSSMANN</u>	GIVEN NAME <u>Wolfgang</u>	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY <u>Hannover</u>	STATE OR FOREIGN COUNTRY <u>Germany DEX</u>	COUNTRY OF CITIZENSHIP <u>Germany</u>
	POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>Feodor-Lynen-Strasse 31</u>	CITY <u>Hannover</u>	STATE OR COUNTRY <u>Germany</u> ZIP CODE <u>D-30625</u>
202	FULL NAME* OF INVENTOR	FAMILY NAME <u>SCHRADER</u>	GIVEN NAME <u>Michael</u>	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY <u>Hannover</u>	STATE OR FOREIGN COUNTRY <u>Germany DEX</u>	COUNTRY OF CITIZENSHIP <u>Germany</u>
	POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>Feodor-Lynen-Strasse 31</u>	CITY <u>Hannover</u>	STATE OR COUNTRY <u>Germany</u> ZIP CODE <u>D-30625</u>
203	FULL NAME* OF INVENTOR	FAMILY NAME <u>STAENDKER</u>	GIVEN NAME <u>Ludger</u>	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY <u>Hannover</u>	STATE OR FOREIGN COUNTRY <u>Germany DEX</u>	COUNTRY OF CITIZENSHIP <u>Germany</u>
	POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>Feodor-Lynen-Strasse 31</u>	CITY <u>Hannover</u>	STATE OR COUNTRY <u>Germany</u> ZIP CODE <u>D-30625</u>

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201*	SIGNATURE OF INVENTOR 202*	SIGNATURE OF INVENTOR 203*
<u>Wolfgang Forssmann</u>	<u>Michael Schrader</u>	<u>Ludger Staendker</u>
DATE <u>20. Oct. 1998</u>	DATE <u>25 October 1998</u>	DATE <u>20/10/1998</u>

☐ Additional inventors are named on separately numbered sheets attached hereto  
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**JACOBSON, PRICE, HOLMAN & STERN**  
**ADDITIONAL INVENTORS**

\* Inventor(s) name must include at least one unabbreviated first or middle name

204	FULL NAME * OF INVENTOR	FAMILY NAME <u>RAIDA</u> 4-00	GIVEN NAME <u>Manfred</u>	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY <u>Hannover</u>	STATE OR FOREIGN COUNTRY <u>Germany</u> <u>DEX</u>		COUNTRY OF CITIZENSHIP <u>Germany</u>
	POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>Feodor-Lynen-Strasse 31</u> <u>Hannover</u>		STATE OR COUNTRY <u>Germany</u>	ZIP CODE <u>D-30625</u>
205	FULL NAME * OF INVENTOR	FAMILY NAME <u>SCHULZ-KNAPPE</u> 5-00	GIVEN NAME <u>Peter</u>	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY <u>Hannover</u>	STATE OR FOREIGN COUNTRY <u>Germany</u> <u>DEX</u>		COUNTRY OF CITIZENSHIP <u>Germany</u>
	POST OFFICE ADDRESS	POST OFFICE ADDRESS <u>Feodor-Lynen-Strasse 31</u> <u>Hannover</u>		STATE OR COUNTRY <u>Germany</u>	ZIP CODE <u>D-30625</u>
206	FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY		COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
207	FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY		COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
208	FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY		COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
209	FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY		COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
210	FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY		COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE
211	FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OF FOREIGN COUNTRY		COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 204*	SIGNATURE OF INVENTOR 205*	SIGNATURE OF INVENTOR 206*
DATE <u>20/10/1998</u>	DATE <u>15/10/1998</u>	DATE
SIGNATURE OF INVENTOR 207*	SIGNATURE OF INVENTOR 208*	SIGNATURE OF INVENTOR 209*
DATE	DATE	DATE
SIGNATURE OF INVENTOR 210*	SIGNATURE OF INVENTOR 211*	
DATE	DATE	

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